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Mammal species inventory using various trapping methods in Zone 4 of Billy Barquedier National Park, Belize during rainy season

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Abstract

Belize is a small country, but it is extremely ecologically diverse. Based on the few studies conducted in Belize, the abundance of mammals is low but diversity is high. Particular findings note the number and identity of species differed between four sites in the Maya Mountains of Belize, indicating that a data set from a single site is not representative of the Neotropical region. Insufficient data is available to estimate current species richness of many areas in Belize, including Billy Barquedier National Park (BBNP). The objective of this study was to explore trapping and documentation methods of terrestrial mammals in BBNP, particularly in Zone 4, and to provide a baseline study of the present species. To accomplish the objectives, four methods were used: 1) direct visual observation; 2) observation of animal tracks; 3) live traps; and 4) game cameras. As expected based on previous studies, endangered species were present amongst the 16 mammal species documented. The various documentation methods presented unique biases towards species, with game cameras capturing the greatest mammal diversity. Further monitoring of animals in BBNP is needed for more accurate information regarding species richness and biodiversity. A controlled, consistent, long-term assessment of the number and composition of mammal species within BBNP could potentially improve management practices and conservation efforts.

Introduction

Biodiversity Loss and Impact + Conservation

Approximately 9 million types of plants, animals, protists, and fungi live on Earth (Cardinale, Duffy, Gonzalex, Hooper, Perrings, Venail . . . Sveriges 2012). Furthermore, these living organisms all function in ecosystems and contribute to meeting human needs with goods and services (Cardinale et al., 2012). Diverse ecosystems are more productive; therefore, increased biodiversity results in increased efficiency and resource richness (Cardinale et al., 2012). The first Earth Summit took place in 1992, and the majority of nations declared acts by humans were diminishing Earth's ecosystems, genetic variation, species, and biological traits (Cardinale et al., 2012). This claim led to questions about how the loss of biological diversity will affect the ecosystems and their ability to provide the goods and services that society utilizes (Cardinale et al., 2012). Research dating back to the 1980s unveiled some of these consequences and found certain life forms could substantially alter the structure and function of whole ecosystems: habitat structure, productivity, nutrient recycling, and more (Cardinale et al., 2012). After the Earth Summit, major research initiatives developed across the world, and 600 experiments were published by 2009 resulting in unequivocal evidence that biodiversity loss reduces the efficiency of ecosystems to capture essential nutrients, produce biomass, and decompose and recycle biologically essential nutrients (Cardinale et al., 2012)

Species around the world are still disappearing, and many claim the phenomenon is the sixth mass extinction on Earth (Ceballos, Ehrlich, Barnosky, Garcia, Pringle, & Palmer, 2015). Changes in ecosystem components, loss of species in the food web, and the introduction of new species all result in environmental deteriorations (Hakim, 2017). In 2002, world leaders committed to significantly reducing the rate of biodiversity loss by 2010, but a study in 2010 showed the rate of biodiversity loss continued over the previous four decades and did not appear to be slowing (Butchart, Walpole, Collen, Strien, Jorn, Scharlemann, Rosamunde, Almond, . . . Watson, 2010). Declines in biodiversity were still prevalent with no significant reductions in rate, and biodiversity pressures increased (Butchart et al., 2010). Despite some conservation achievements, efforts to address biodiversity loss need to be strengthened substantially (Butchart et al., 2010). The consequences of biodiversity loss are significant, and in order for conservation efforts to be effective, the elements of biodiversity must be understood (Turner, 2014). Some of these elements include conditions of ecosystems, the number and identities of species, and how they change over time (Turner, 2014). Coherent global biodiversity monitoring is essential for tracking and improving the effectiveness of conservation efforts (Butchart et al., 2010).

Role of Mammals in Ecosystem

Evidence indicates that there have been steady declines in populations of specifically vertebrates (Butchart et al., 2010). Much work has shown that loss of higher consumers (often mammals) can cascade through a food web; the loss of one top predator species can alter vegetation structure, fire frequency, and even disease epidemics across ecosystems (Cardinale et al., 2012). A study in 2011 stated that, since the 1500s, 76 species and seven subspecies of mammals have gone extinct and another two only existed in captivity (Visconti, Pressey, Giorgini, Maiorano, Bakkenes, Boitani, . . . Rondinini, 2011). Medium-to-large mammals represent a rich and functionally diversified component of the biome, but they continue to be threatened by hunting and habitat loss (Rovero, Martin, Rosa, Ahumada, & Spitale, 2014). To track changes in these populations, studying species' richness and composition is required first (Rovero et al., 2014). Understanding and conserving a species begins with a detailed biological survey of the biological and ecological aspects of the targeted species (Hakim, 2017).

Ecotourism

Species and ecosystem diversity are important components in tourism, and particularly birds, reptiles, and mammals have become famous tourist attractions (Hakim, 2017). There is a significant role for the biological field in tourism industry development (Hakim, 2017). Threats to biodiversity exist in nature-based tourism, including pollution, exotic plant species invasion, habitat changes and degradation, habitat loss, and wildlife disturbance; therefore, the growth of tourism in biodiversity-rich areas has a major impact on the growth or decline of biodiversity (Hakim, 2017). In nature-based tourism, managing biodiversity for sustainable and competitive development requires basic understanding of the principles of biology, and scholars point out that supporting biodiversity conservation in these destinations is a fundamental responsibility (Hakim, 2017). Unfortunately, an understanding of biology is often lacking in tropical developing countries where ecotourism is rising (Hakim, 2017). Biology is more than the simple principles of living systems, and complex biological concepts are important in the recent spread of eco-industries (Hakim, 2017). Linking ecotourism and species conservation requires data on species diversity and characteristics, population structure, and how species and their environment interact (Hakim, 2017). Identifying and mapping biodiversity is an important component of developing sustainable ecotourism, and a failure to do so may lead to mismanagement of biodiversity and consequences from biological system disturbances (Hakim, 2017).

Biodiversity Loss and Ecotourism in Developing Countries

The need for data on distribution and abundance of tropical species requires tropical mammal community assessments (Rovero et al., 2014). A study in 2011 found that, with some exceptions, most countries with the largest predicted losses of suitable habitat for mammals are in Africa and the Americas. Most of the countries with future hotspots of terrestrial mammal loss do not align with present global conservation priorities (Visconti et al., 2011). Agriculture and grazing have recently expanded almost exclusively in the tropics with approximately a 7 million ha increase of cattle pastureland in Central America between 1980 and 2000 (FAO, 2017). Globally, between 1995 and 2007, agricultural land increased by 400 million ha in developing countries, and this agricultural expansion continues as the demand for food and products increases (FAO, 2017). Local conservation efforts in developing countries may not be enough to prevent the loss of mammals as human populations and product consumption grow (Visconti et al., 2011). Policies for biodiversity conservation and prevention of initial pressures are required in these developing communities (Visconti et al., 2011). A study focused on achieving biodiversity-sensitive communities stated that effects of urbanization on wildlife extend into adjacent habitats: Retaining large, undisturbed areas away from development and avoiding intensive development adjacent to conservation areas is necessary to maintain biodiversity (Ikin, Le Roux, Rayner, Villasenor, Eyles, Gibbons, . . . Lindenmayer, 2015). The study also indicated biodiversity education programs and engagement of locals in conservation efforts improve local support and overall outcomes (Ikin et al., 2015). Gurung and Seeland examined the benefits of ecotourism as a sustainable means of development in a developing community of India (2011). They examined sources of livelihood, the impact of tourism, and the readiness of rural communities to participate in income-generating activities, and the results showed that 64% of the households received economic benefits from tourism; thus, ecotourism may be a component in sustainable development of tropical communities (Gurung & Seeland, 2011).

Belize

Belize is an example of a country that often attracts ecotourists with inland rainforests, a coastal environment, and the barrier reef (Blersch & Kangas, 2013). Belize is a small country, but it is extremely ecologically diverse (Young, 2008). With a population of only 354,000 people, tourism is the major source of income (CIA, 2017), but if the biodiversity in Belize is to be maintained, the country must utilize best-management practices driven by applied scientific research (Young, 2008). Conservation is threatened by deforestation rates, improper solid waste management, rapid coastal development, increasing poverty, weak institutional and legal frameworks, and the recent discovery of "sweet" crude oil (Young, 2008). In addition, illegal harvesting of flora and fauna, illegal hunting, illegal logging, looting of the extensive cave system, and translocating of invasive species all challenge conservationists and institutions (Young, 2008). Unless Belize is able to respond appropriately and quickly, the environment that drives much of Belize's economy will suffer (Young, 2008). Some initiative to conserve natural resources exists in Belize, but the country lacks the required research infrastructure and in-country scholarship to act effectively (Young, 2008). Scientists from developing countries, particularly Belize, face absence of a culture of conducting research and lack research programs (Young, 2008). Most of the research conducted and published for Belize has been conducted by foreigners because it is difficult for Belizean scientists to compete internationally for research grants (Young, 2008). Scientists interested in terrestrial ecosystems find it particularly difficult to secure funding for applied conservation-oriented research, so Young suggests forging collaborative research opportunities with research-focused institutions in the United States (2008). Twelve terrestrial mammals of Belize (excludes marine mammals and bats) appeared on the National List of Critical Species by J. C. Meerman in 2005, and, according to IUCN, approximately eleven remain endangered, vulnerable, or near threatened (2018). Few terrestrial animal studies have been conducted in Belize, but some of the regions explored for terrestrial mammal studies include the Chiquibul Rainforest (Engilis, Cole, Caro, 2012), the Raspaculo

River Basin and Cockscomb Basin Wildlife Sanctuary (Caro, Kelly, Bol, Matola, 2001), and the Bladen Nature Reserve (Klinger, 2006).

In 2001, a small mammal study was conducted in the Chiquibul Forest Reserve, located in western Belize in the Maya Mountains (Engilis, Cole, Caro, 2012). The Chiquibul Forest Reserve is west of Billy Barquedier National Park in a district called Cayo (Engilis, Cole, & Caro, 2012). The study included 3,686 trap-nights capturing 154 specimens and representing 15 species of small mammals (Engilis, Cole, & Caro, 2012). Five sites were sampled where small mammal habitats were likely to occur (Engilis, Cole, & Caro, 2012). Caro, Kelly, Bol, and Matalo extended the study to multiple sites in the Maya Mountains of Belize, including the Chiquibul Forest Reserve, Raspaculo River Basin, Bladen Nature Reserve, and Cockscomb Basin Wildlife Sanctuary (2001). The objective was to obtain a more representative set of data for the Maya Mountain region (Caro et al., 2001). Klinger subsequently examined the impacts of natural disturbances, such as floods and hurricanes, on the distribution and abundance of small mammal species in Bladen Nature Reserve (2006). Caro, Kelly, Bol, and Matalo indicated the abundance of small mammals in the Maya Mountains is low but diversity is high (2001). An important aspect of their study was that number and identity of species differed between sites in the Maya Mountains; less than 33% of species were found in all four sites and greater than 20% were found in only one area (Caro et al., 2001). This indicates that a data set from a single site is not representative of an entire Neotropical region (Caro et al., 2001). In total, 51 terrestrial mammals were documented in the four sites in this study, and of these, some may or may not appear in other protected areas with similar terrain, climate, and close proximity (Caro et al., 2001). Published researchers that have conducted terrestrial mammal studies in Belize discuss the need for further studies in varying sites, and relatively few studies on these species in Belize are currently available (Engilis et al., 2012; Caro et al., 2001; Klinger, 2006).

Billy Barquedier National Park

Insufficient data is available to estimate current species richness of many regions of Belize (Caro et al., 2001); one of the regions lacking data is Billy Barquedier National Park (BBNP) in Stan Creek District with primary entrances in Steadfast (*Figure 1* and *Figure 2* depict BBNP location on maps). The park is a 660-hectare wildlife reserve that is divided into four management zones as follows: Zone 1 (recreation and education zone); Zone 2 (camping and environmental zone); Zone 3 (protection zone); and Zone 4 (strict conservation zone) (STACA, 2017).

Although STACA and Forest Department of Belize manage and protect the land, there is little published evidence of the mammal species protected within the park (STACA, 2017). Documenting the variety of species and the species richness is the first step to understanding biodiversity of the area (Turner, 2014). Data on the variety of mammal species in the park would feasibly educate the community, provide information for determining species richness and biodiversity, and improve the ecotourism in the area (Ikin et al., 2015; Turner, 2014; Young, 2008; Gurung & Seeland, 2011). Protection of the park could potentially preserve components of earth's biodiversity (Visconti et al., 2011), whereas improvement of ecotourism in Stan Creek District may provide increased profits for locals and growth in the economy (Gurung & Seeland, 2011). The objective of this study was to explore trapping and documentation methods of terrestrial mammals in Billy Barquedier National Park, particularly in Zone 4, and to provide a baseline study of the present species. To accomplish the objectives, four methods were used: 1) direct visual observation; 2) observation of animal tracks; 3) live traps; and 4) game cameras.

This novel study was necessary prior to conducting further biodiversity and ecological research. If endangered species are present in the park, as expected based on previous studies in Belize, accurate assessments of the number and composition of species are essential for proper conservation and management practices (Colwell & Coddington, 1994).

Limitations

Four methods are used for the study because each has limitations. Detection rates often vary considerably among species due to differences in abundance and behavior and in the ability of observers to identify species (Boulinier, Nichols, Sauer, Hines, & Pollock, 1998). Measuring a complete census is feasible only for plants and very few mammals (Colwell & Coddington, 1994). Even for groups that are more feasibly assessed, measuring means sampling in most cases because collecting all species is notoriously difficult either to attain or monitor (Colwell & Coddington, 1994). Dorazio and Royle explain that samples of communities are unlikely to reveal every species and usually end with incomplete coverage and imperfect detection (2005). Although there is imperfection, analysis of camera trap data and other data sources with account for imperfect detection can provide a helpful ecological assessment of mammal communities (Rovero et al., 2014). Regardless of the great abundance of mammals, or the lack thereof, a novel study of the mammals in Billy Barquedier National Park is one step toward conservation of the earth's biodiversity and all of the resources humans acquire from it.

Literature Review

Challenges of Sampling and Complete Census

Mammals represent a rich and diverse component of the biome, but they are threatened by hunting, habitat loss, and a host of other factors (Rovero et al., 2014). Reiterating Colwell and Coddington (1994), the conservation and management of biodiversity requires accurate assessments of the number and composition of species. This remains in the literature throughout time: Assessing communities of mammals requires thorough studies of species' richness and composition, and to discern shifts in distribution and habitat, species abundance must be acquired (Rovero et al., 2014). Changes in species abundance can impact a spectrum of ecosystem processes (McShea, Forrester, Costello, He, & Kays 2016).

Although studies strive to document patterns of abundance and community structure, there is a lack of data that is appropriately scaled and accurate (McShea et al., 2016). During these studies, detection rates among species are extremely variable due to differences in abundance, body size and mass, life history, behavior, habitat preferences, and the observers' abilities to identify the species (Dorazio & Royle 2005; Carvalho et al., 2016). Multiple methods of data collection are necessary to acquire adequate mammal inventories (Carvalho et al., 2016). Colwell and Coddington (1994) stated that a complete census is notoriously difficult to attain, and this remains true in modern research (Caro et al., 2001; Harmsen et al., 2010; Rovero et al., 2014; Carvalho et al., 2016; Kolowski & Forrester 2017). Because of these challenges, samples are used to estimate the number and composition of species, but samples are unlikely to represent complete coverage or perfect detection that reveals every species (Dorazio et al., 2005).

Different sampling methods and designs result in biases toward different species, so it is important to assess detection probabilities and correct (or account) for those biases (Harmsen et al., 2010). The study objective and target mammal species will influence which methods will have the greatest accuracy, capture success, and probability of correct identifications (Carvalho et al., 2016). Detection often consists of frequencies of footprints, burrows, markings, direct observations, and photo captures along trails and transect lines, but the assumption of equal detection probabilities is rarely valid (Harmsen et al., 2010). Even if all methods are similarly adequate, each methodology has a distinct cost-efficiency relationship with variable costs, time commitments, and skill requirements (Carvalho et al., 2016), so any given method or combination of methods presents barriers.

Cusack, Dickman, Rowcliffe, Carbone, Macdonald, and Coulson (2015) assessed relative changes in species rankings between two camera surveys in the same simultaneous setting and found that some species showed significant shifts in rank from one survey to the next. A case study in Belize that focused on the implications found that relatively subtle differences in behavior can still cause differences in detectability even for similar sized species occupying similar niches (Harmsen et al., 2010): For example, the willingness of some species to use exposed areas will affect the probability of capturing those species along forest trails or transects. Small neotropical mammals, like the paca and armadillo, prefer dense undergrowth, whereas larger mammals, such as jaguars and pumas, walk on open trails (Harmsen et al., 2010). Even with the existing biases, researchers utilize transects and trails to survey wildlife in dense forests and vegetation because researchers are restricted in movement off trail (Harmsen et al., 2010). The great impact of imperfect detection on results has been known for decades and has driven development of a variety of statistical methods for estimating the number of distinct species in a community (Dorazio & Royle 2005). According to a case study in Belize, comparisons of relative abundance indices between species should be made with caution and should account for trail width, habitat types, habitat openness and other factors (Harmsen et al., 2010). Without accounting for these variables, capture rates may not be an appropriate index of relative abundance (Harmsen et al., 2010).

In addition to accounting for these variables within a site, a mammal inventory of multiple sites in Belize found that restricting a study to a single research camp will not adequately represent a list of species of the entire locality (Caro et al., 2001). Rare species, species with large home ranges that visit the site infrequently, species with specific habitat requirements, and competitively weak species may go undocumented; so Caro et al., (2001) advocated that research efforts be geared toward sampling multiple sites, rather than simply increasing time spent at one site.

One can conclude that previous publications on taking inventories of mammals, particularly in Belize, call for a variety of data collection tools and survey sites to obtain a complete list of species (Dorazio & Royle 2005; Harmsen et al., 2010; Caro et al., 2001). Biases are present in all forms of data collection, so researchers must account for imperfect detection prior to making inferences about species richness and relative abundance (Dorazio & Royle 2005; Harmsen et al., 2010; Caro et al., 2001). The challenges of surveying mammals, as outlined previously, ultimately support the need for further studies in all Neotropical regions.

Utilizing Direct Observation and Animal Tracks

Neotropical mammals are not easily observed in their habitats, and few studies compare the efficiency of methods designed to register mammals' tracks (Olifiers, Loretto, Rademaker, Cerqueira, 2011). Two experiments in Brazil compared artificial methods (sooted paper and plastic board) with sand plots using track presence/absence, total number of tracks, and number of identifiable tracks (Olifiers et al., 2011). They used many different baits from bacon and meat to seeds and fruit, and they set up track stations in many different habitats (Olifiers et al., 2011). Eleven medium- to large-sized mammal species were identified with 173 tracking station-nights (Olifiers et al., 2011). The results showed evident differences between methods, with sand plots outperforming artificial methods in 2/3 of the comparisons, and individuals were generally reluctant to step on plastic board or sooted paper (Olifiers et al., 2011). The use of artificial materials to register mammal tracks resulted in underestimates that are especially relevant to short-term ecological studies because species tend to need time to get used to artificial methods (Olifiers et al., 2011). This can be explained by mammals' outstanding sense of smell or sight that allows them to perceive artificial materials in the environment (Olifiers et al., 2011). Olifiers et al., (2011) recommended traditional sand plot methods and avoidance of artificial materials for short term studies, but also claimed artificial methods may be useful under specific conditions or more comprehensive sampling efforts. The aversion of mammals due to artificial materials may exist for all methodologies that introduce foreign materials in the design (Olifiers et al., 2011).

Harmsen et al. (2010) conducted a study to analyze detection probabilities of a range of neotropical mammals on trials in dense forests using camera traps and track data. Footprints of Central American mammals (weighing less than 2 kg) are easily identified by tracks from their size and shape (Harmsen et al., 2010). During the study in Belize, all surveyors could identify well-defined footprints to species level, but tracks lacking sufficient definition for identification to species level were discarded from the data (Harmsen et al., 2010) Other important contributions to the literature from the study were analyses of animals' tendencies to follow or cross trails, which impacts detection probability based on camera or trap placement. They surveyed trails, analyzed camera captures along trails, and noted the direction of travel of specific species. Some of their findings were that tapir tracks were only found following trails, whereas pacas, opossums, armadillos, and agoutis never followed trails for more than 100 m. Identifying tracks along trails may be a feasible means of identification in the Neotropical regions based on this study's findings, but some mammals have a much lower probability of detection due to their tendency to follow or cross trails (Harmsen et al., 2010).

A study in Belize simultaneously monitored medium and large mammals along five trails between July and August 2009 and January and February 2010 (Carvalho et al., 2016). The study focused on three different sampling methods commonly used to monitor mammals in seasonal tropical forests, including camera traps, line transects for direct observation of animals, and line transects seeking tracks/footprints (Carvalho et al., 2016) The researchers then analyzed species richness detected by each method and quantified their cost-efficiencies (Carvalho et al., 2016). Carvalho et al., (2016) explained that direct observations were best suited for collecting data on arboreal species in the study region because they seldom descend from the treetops or move along trails, which indicates why primates recorded by direct observation were not recorded by the other two methods. Seeking tracks and using game cameras recorded terrestrial species that move long distances and have nocturnal and elusive habits or occur in low density (Carvalho et al., 2016). Complementary use of direct observation and tracks/footprints, in conjugation with camera traps, will enhance identification accuracy, may allow individual identification, and will, therefore, permit more accurate abundance estimates (Carvalho et al., 2016). Although the combination is the most effective, direct observation and searching for tracks/footprints requires experienced field technicians and a greater number of researchers (Carvalho et al., 2016). In this study, tracks/footprints and direct observations contributed more detected species, but camera trapping did detect a species that the other two methods did not register. The continuous sampling of camera traps may counterbalance the time restriction of observing animals or tracks in some ways (Carvalho et al., 2016).

Utilizing Game Cameras

Camera trapping is a common tool for wildlife ecologists, especially for studies that draw data from photo capture rates or presence/absence of information (Kolowski & Forrester 2017).

Camera trapping has been presented as an efficient way to inventory mammal species in tropical communities by numerous studies (Rovero et al., 2014). Camera traps have infrared sensors that detect movement and trigger the camera to take a photo, a sequence of photos, or a video clip (McShea et al., 2016). Using these photos or clips, researchers obtain records of wildlife in specific locations, dates, and times, and they can then quantify distribution across a landscape (McShea et al., 2016). Many of the surveys that utilize camera trapping exclusively target features of the landscape to increase probability of photographing one or several focal species (Cusack et al., 2015). Issues with drawing inferences on mammal richness, composition, and structure arise when studies ignore the biases in species detection when sampling only a limited set of potential habitat features (Cusack et al., 2015). Non-random camera trap placement violates a principle of random selection of sampling units (Cusack 2015). Camera traps are a powerful tool for surveying mammal communities though (Kolowski & Forrester 2017). The factors influencing detectability are poorly understood, and the better researchers understand that the more accurately camera traps can model abundance, diversity, and species interactions (Kolowski & Forrester 2017). The effect of camera placement has been assessed by multiple studies and is becoming one of the more well-known impacts on data, but even these biases are not well understood (Kolowski & Forrester 2017).

One study explored the influence of strictly random versus strictly trail-based placement strategies on the observed richness, composition, and structure of terrestrial mammal communities (Cusack et al., 2015). Trails may be cost-effective patrolling routes, and areas close to rivers may be gathering places for herbivores during dry seasons (Cusack et al., 2015). The researchers in the random vs. trail study compared richness, composition, and structure of the two observed communities. Placement strategy did not affect overall community composition and structure but influenced community richness and capture rates of specific species, especially carnivores and other larger mammals (Cusack et al., 2015). Despite similarities, neither random placement nor trail-based placement recorded all 41 species detected overall, so neither offered a completely optimal design for surveying mammals (Cusack et al., 2015). The study's conclusions suggested that placement strategy is unlikely to affect inferences made at the community level of forested habitats when extensive surveys of an average of >1,400 camera trap days are implemented (Cusack et al., 2015). If sampling days are less extensive, trail-based camera placements may more rapidly detect more species (Cusack et al., 2015).

Another study found that species accumulation occurred faster for cameras with log features in view, but confidence intervals for species richness began to level out after approximately 659 camera nights (Kolowski & Forrester 2017). An additional experiment for this study showed that cameras placed on game trails accumulated unique species quicker than control cameras off trail, but richness values overlapped after approximately 385 nights of effort (Kolowski & Forrester 2017). Despite the overlap that develops with extensive data collection nights, small-scale factors can have significant impacts on detection when camera traps are used (Kolowski & Forrester 2017). Kolowski and Forrester (2017) suggested incorporating the presence and quality of surrounding features into analytical procedures or controlling for these features in the study design, and they stress that more than 650 camera nights (a relatively large amount of effort) were needed to remove biases.

Harmsen et al., (2010) reiterated the need to correct for biases with information about the relation between capture probability and other variables at a camera location. The study tested the assumption of equal detection probabilities of two ecologically similar predator species (jaguars and pumas) and prey species in the rainforests of Belize. With 110 camera stations at 1 km intervals, the study sampled a wide range of microhabitats and trail types. Higher capture rates of jaguars, pumas, and ocelots were strongly associated with wider, established trail systems (Harmsen et al., 2010). Capture rates for the red brocket deer decreased as trail width increased, and pacas and tapirs were photographed more on recently cut trails rather than established trails (Harmsen et al., 2010). Most other mammals were not strongly correlated with trail variables, but tapirs and opossums were associated with closer proximity to rivers (Harmsen et al., 2010). Off- and on-trail cameras detected different species too. Capture rates of red brocket deer, pacas, and tapirs were high in off-trail locations, while jaguars and pumas were captured more frequently on-trail (Harmsen et al., 2010). Even these two very similar cats with similar ecological niches differed in trail use, as evidenced by pumas following trails more completely and jaguars deviating from the trails; as a result, Harmsen et al., (2010) concluded photographic captures and tracks along trails may not be appropriate for comparison between Neotropical species (Harmsen et al., 2010). Although assumptions about community structure and abundance may not be accurate with camera traps, photographs do allow researchers to compile a baseline list of species with higher precision in terms of species identification (Kolowski & Forrester 2017; McShea et al., 2016; Carvalho et al., 2016; Cusack et al., 2015; Rovero et al., 2014; Harmsen et al., 2010).

A study in the tropical forests of Tanzania proposed an example of a baseline assessment of species' occupancy (Rovero et al., 2014). The researchers used 60 camera locations, cumulated 1,818 camera days, and yielded 10,647 images of 26 species of mammals. The study concluded that camera trap data, with account for imperfect detection, provided a solid ecological assessment of mammal communities (Rovero et al., 2014) Suggestions for increasing species detection rate were increasing number of cameras within the study area, using different camera positions, and, when limited numbers of cameras are available, relocating cameras to new sites rather than sampling the same sites over a longer period (Carvalho et al., 2016). Carvalho et al., (2016) also advised using state-of-the-art cameras to avoid technical problems and to enhance the quantity and quality of data.

Utilizing Live Traps

Traditional mammal sampling techniques, particularly live trapping, are challenging, and various conditions affect efficacy of sampling efforts (DeSa, Zweig, Percival, Kitchens & Kasbohm 2012). DeSa et al., (2012) compared indirect captures and direct physical live-trap captures and compared responses to different baits to find an effective method for sampling small mammals. The study concluded that Sherman traps (more tunnel-like traps with larger openings) were more effective than Fitch traps (traps with a mesh appearance and slightly smaller openings), but there was no apparent relationship between baits (DeSa et al., 2012). The researchers did, however, suggest using scratch feed or sunflower seeds rather than oats or suet because of spoilage rates and cleaning difficulty (DeSa et al., 2012).

A study in Brazil assessed mammal abundance, richness, and community structure using live-traps, pitfalls, and sightings (Cáceres, Nápoli, Casella, Hannibal 2010). Seventeen small mammal species and fifteen large mammal species were documented with the combination of collection techniques (Cáceres et al., 2010). Although live trapping involves vigorous and skilled technicians, the study recorded adequate data to assess the communities (Cáceres et al., 2010).

Two of the most influential works in Belize mammal biodiversity are a list of medium and large mammals compiled by N. Bol in 1999 and a live trap inventory of mammals in multiple sites across the Maya Mountains in 2001 (Caro et al., 2001). The live trap inventories recorded 51 species of nonvolant mammals in the 4 sites of the Maya Mountains (Caro et al., 2001). The study speculated that abundance of mammals was low, but diversity was high (Caro et al., 2001). This is supported by the differences in numbers and identities of species between sites, but these differences may have resulted from differences in research effort, soils, vegetation, elevation, rainfall, and/or the time in which sampling occurred (Caro et al., 2001). Less than 33% of mammal species were found at all 4 sites, and greater than 20% were found at only one site. For this reason, the researchers stressed the need to study many sites, rather than one camp site, and collect more data before inferences about species richness or community structure in the Maya Mountains of Belize can be confirmed (Caro et al., 2001).

Efficiency of sampling methodology depends on the study objective, mammal species of interest, and the resources available (Carvalho et al., 2016). Every method has limitations and biases, so multiple methods in various placements result in the most diverse collection of data (Carvalho et al., 2016). Imperfect detection alone solidifies the need for more mammal inventories. With such few studies completed in Belize, there is not enough information to make accurate inferences or estimates of mammal biodiversity or even confirm a thorough baseline list of the mammals in the country (Caro et al., 2001; Harmsen et al., 2010).

Methodology

Design, Purpose, Objectives

This qualitative study utilized a nonexperimental descriptive design. The purpose of this study was to explore trapping and documentation methods of terrestrial mammals in Billy Barquedier National Park, particularly in Zone 4, and to provide a baseline study of the present species. To accomplish the purpose, the following objectives will be met: 1) utilize 4 methods to document mammal species in Zone 4 (direct observations, animal tracks, live traps, and camera traps); 2) compile inferences and suggestions for surveying Neotropical mammals in Zone 4; 3) assess the mammal community in a broad sense; and 4) analyze the data for species' habitual patterns.

Treatments

Because this study is a non-experimental design, there are no particular treatments included in the methodology. All trapping methods and animal handling procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (protocol no. 17084) prior to travel to Belize and study implementation.

Instruments

Direct Observations:

Direct observations were made on- and off-trail during daylight hours in Zone 4 and on trail in the evenings with the use of flashlights. Field guides were required standard instruments used for identification: *A Field Guide to the Mammals of Central America and Southeast Mexico*, Second Edition (written and illustrated by Fiona A. Reid, 2009) and the *Neotropical Rainforest Mammals A Field Guide*, Second Edition (written by Louise H. Emmons and Illustrated by Francois Feer, 1999) Binoculars were used to see mammals from a distance. If researchers were able, photographs of the animals encountered were captured with either an iPhone 6 or Canon digital camera, stored on standard SD cards, and subsequently uploaded to a laptop.

Direct observations were recorded immediately at the time of observation with the date, time, and coordinates. If feasible, photos were taken, stored on a standard SD card, and uploaded to a laptop. The data utilized from this part of the study include the date, time, coordinates, species, and the behaviors observed (sitting in a tree, walking on the ground, swinging limb to limb, etc.)

Animal Tracks:

A measuring tape was laid alongside any tracks found in the soil, and an iPhone 6 or Canon digital camera was used to photograph the tracks. Photos were stored on standard SD cards and uploaded to a laptop for more thorough viewing. Field guides with detailed track drawings and descriptions (Reid, 2009; Emmons & Feer, 1997) were used to identify animals based off tracks in the soil.

Data from tracks in the soil were collected by placing a measuring tape beside the individual tracks and taking photos with an iPhone 6 or Canon digital camera. The date, time, and coordinates were also recorded; however, the only information used for analysis was species, approximate date and coordinates because there is no accurate measure for when the tracks were made.

Live Traps:

Sherman traps (12) and Havahart traps (2) were used for live trapping. The Sherman traps were aluminum traps (8.9 cm x 7.6 cm x 22.9 cm) that are enclosed on all sides. One door lifts into the trap and clicks into place, and bait was placed inside the trap just beyond a pressure-sensitive plate that sits in the center. The trap was adjusted to be more or less sensitive. For this study, the traps were set as sensitive as possible to capture very small mammals. The Havahart traps were galvanized steel wire traps (30.5 cm x 26.7 cm x 81.3 cm) that have one spring-loaded fully metal door. Although the trap allowed ventilation, one downfall to this trap is that animals are visually exposed to predators. Baits used in both types of traps included oats, mango, mango jelly, peanut butter, apple, banana, mami apple, coconut, tuna, and cat food. Approximately one tablespoon of bait was placed in a single trap. Kitchen gloves were used to handle the traps to reduce human scent left on the traps. Pliers and scissors were used to make adjustments to traps if needed. Bungee cords were used to secure traps in place if needed. Once an animal was captured in a Sherman trap, it was released into a clear tub for observation. A measuring tape was placed beside the animal and photos were taken with an iPhone 6 or Canon digital camera to document the animal. These photos were stored on standard SD cards and transferred to a laptop for further observation. Information from *A Field Guide to the Mammals of Central America and Southeast Mexico* (Reid, 2009) was used to identify any mammals captured.

Data from live traps included the species, date, time, coordinates, type of trap, and bait used, with photo evidence to support these findings. Upon discovery of an animal in a Sherman trap, the small mammal was placed into a clear tub for observation. Photos were taken with an iPhone 6 or canon digital camera, and a measuring tape was laid alongside the animal. The species was determined both first person using a field guide (if feasible) and with further investigation of the photos taken once uploaded to a laptop. Some inferences may be made about the type of bait that attracted the animal, but, with few controls or indications of what time the animal entered the trap, the primary information used for analysis was the species trapped, the coordinates, and the date.

Game Cameras:

The Moultrie M-880 Mini Game Camera in Camo with 8.0 Megapixel image sensor and infrared motion sensor was used for all the camera traps in this study (Product Name MCG-M880, Model MCG12594; manufacturer information). The camera has a 4 fps scan rate and 1 second trigger speed, and it was set to take multiple shots for each motion detected (3 consecutive photos with a 5 second time lapse before the next detection and trigger). Game cameras come equipped with straps to wrap around trunks of trees or other objects to position or stabilize the cameras. The camera requires 8 AA batteries, and batteries were changed when below 15% battery life, as indicated by the battery percentage depicted on the game camera screen. Bungee cords were also used, as needed, to secure the cameras in desired positions. Each photo was stamped with the date and time the photo was taken and stored on 16-GB SD cards. The baits were placed in the view of some game cameras to increase the likelihood of documenting mammals and increase the quality of photos taken as animals pause in the lens' view. Photos were uploaded to a laptop weekly, so the SD cards could be cleared for the following week. Photos were examined thoroughly on the laptop to note any animals present in the photos, and *A Field Guide to the Mammals of Central America and Southeast Mexico* (Reid, 2009) was used to identify species in the photos.

Some data from the camera trap method were collected directly by the game cameras. For any photo with a mammal in the frame, the coordinates, bait used (if any), and the species if distinguishable were recorded. Some species were determined easily based on physical appearance, but some required small details to identify the animal to species level. In those cases, the species was not determined until the photos were uploaded to a laptop and multiple field guides were referenced. Photos taken with no mammals present in the frame, as well as unidentifiable animals, were excluded from the records. Because a single animal may have been photographed many times by the same camera, a criterion based on other literature was used to define an independent animal capture. Photo series from the same camera were considered independent animal captures if one of the two following criteria was met: 1) 10 minutes passed with no captures of the same respective species or 2) the photographed animals were easily discernable by visual characteristics (Kolowski & Forrester 2017).

Prior to Analysis

All the species names, dates, coordinates, and times were entered into an Excel sheet before analysis was completed. As previously stated, some methods did not acquire the time an animal was present but indicated a species was present at a particular location. If the time was accurately acquired, the data was categorized into a three-hour time frame to assess activity rhythm.

Data Analysis

Data analysis included primarily descriptive statistics to describe the prevalence of captures of each species. In addition, correlational statistics were used to determine relationships between captures of specific species and the time of day. Distribution data were analyzed in the frequency procedure of SAS (SAS Inst. Inc., Cary, NC). A *P*-value of 0.05 was required for significance in all analyses. The study supplies a list of mammal species in Zone 4 of Billy Barquedier National Park, information regarding mammal behavior, and a broad perspective addressing efficiency of mammal inventory methods in the context of this study.

Results

Sixteen species were identified in Zone 4 of Billy Barquedier National Park (See *Table 1*). Among these species, the margay (*Leopardus wiedii*) is a near threatened species, whereas the Baird's tapir *(Tapirus bairdii)* and the Yucatan black howler monkey (*Alouatta pigra)* are endangered species (IUCN Red List, 2019).

Three species (the Deppe's squirrel, kinkajou, and howler monkey) were identified by visual observations, with the kinkajou and howler monkey being documented solely by direct observation due to their arboreal behaviors. Photos were taken of these species, but the quality of the kinkajou photos was poor because these nocturnal animals were only documented at night.

Tracks indicated the presence of some medium to large mammal species, but all tracks were confirmed by an alternative method except the tracks of the red brocket deer. Red brocket deer tracks were identified along the trail outside of the Zone 4 boundary line and along the bank of Peter Whyte Creek. The endangered Baird's tapir left multiple tracks along the human paths and created large cleared paths within Zone 4.

A total of 135 identifiable photo captures were documented by game cameras following >160 camera trap nights (See *Graph 1*). Chi-Squared analysis indicated a significant difference in photo capture frequency between species (See *Graph 1*). Photo capture frequency versus time revealed a statistical difference between photo captures within 3-hour time intervals throughout the day. The highest frequency of photos was recorded from 2100 to 0300 (86 of 135 photo captures; $p<0.001$), but there was no statistical difference between 2100 to 2400 and 0001 to 0300 (*Graph 2*). Frequency versus observation days suggested that most photos were taken within the first four days of camera placement, but cameras were often relocated within one or two camera trap nights, which may explain the deficit following day 4 (*Graph 3*). Other cameras remained in one location for up to 13 days. Another factor may have been dilution or loss of bait over time. The greatest number of photos were taken on day one of camera placement when bait was most potent ($p=0.003$). Three large cats (the ocelot, margay, and puma) were documented by game cameras. Paca and nine-banded armadillo were most frequently photographed by game cameras of all species ($p<0.001$). Although the animals were not marked and may appear in multiple photos throughout the study, approximately 77% of camera locations captured the paca in identifiable photos, while 73% of camera locations captured the nine-banded armadillo in photos. For examples of game camera photos and to view some of the documented animals, see *Figures* 3-12.

Only one species (hispid cotton rat) was identified by live trapping, suggesting inefficiency in using this method in Zone 4 of BBNP. See *Figure* 13 for documentation photos.

Conclusions/Discussion

The methodology used for setting many of the cameras focused on locating a trail created from animal traffic and setting the camera to capture the trail in the frame. Mami apple was a frequently used bait because most of the neotropical mammals in Belize eat the fruit. This may have created a bias for the habitually traveling animals and animals that are strongly attracted by the mami apple; however, cameras were also periodically placed with larger frame shots, with different bait, and away from small animal trails. Photos of pacas and armadillos prevailed regardless of camera location or bait, totaling 43 identifiable photos of paca and 58 identifiable photos of nine-banded armadillos within Zone 4. Moreover, the evidence may suggest that large populations of both species occupy Zone 4 of Billy Barquedier National Park. If the populations are large, as expected, Zone 4 of Billy Barquedier National Park would be a suitable area to further investigate these species in the Neotropical region.

Live trapping methods were not effective for data collection in Billy Barquedier National Park. Mango, mami apple, peanut butter, oats, and tuna-flavored cat food were all used in trials. On each trap night, 12 small Sherman traps and 1-2 large Havahart traps were baited and set. A number of variations in live trapping were employed: covering the exterior of the traps with dried leaves; layering the bottom of the traps with dirt and/or leaves; leaving the traps untouched for multiple nights to decrease the human scent; wearing gloves while baiting the traps; setting the traps near water; setting the traps in piles of fallen limbs or brush; and setting the traps along animal trails. All methods were unsuccessful in capturing species in the live traps. Game cameras were used on a couple occasions to observe activities around traps and determine how animals behave near the traps. Few photos were taken and little information regarding live trapping was collected. However, one species, hispid cotton rat (*Sigmodon hispidus)*, was captured in a Sherman trap using canned tuna as bait with placement parallel and adjacent to a fallen log. There is not enough evidence to make suggestions for more effective live trapping in Zone 4 of Billy Barquedier National Park. Further research is necessary to improve the efficiency of live trapping for research purposes.

Of the methods employed, game cameras provided the greatest number of unique terrestrial mammal species. Conversely, first-person encounters (direct observations) were the only successful method for documenting arboreal species, such as kinkajous and howler monkeys. The current study was limited to Zone 4; however, a colleague Kelsey Johnson replicated the study design in Zone 1 and separately reported her findings. Zone 2 and Zone 3 were entirely excluded from the mammal inventory, and the duration of the study was constrained to mid-June through late-July. Ideally, future studies will include all four zones of Billy Barquedier National Park with significantly more trap nights. Although the objective of the study was simply to compile a baseline list of species, more consistent survey methods with thorough records would potentially provide stronger conclusions regarding species richness and ecological behavior. Examples of more controlled study designs could feature grid placement of traps and cameras or treatments with varied baits. Due to lack of prior research experience related to Neotropical mammal species, other experts and researchers are encouraged to request documentation and scrutinize the identities of the listed species.

Tables

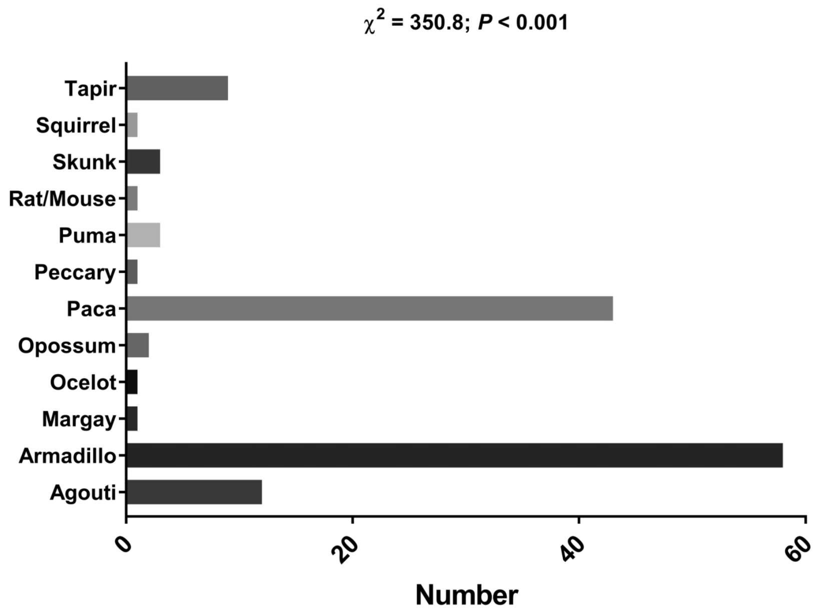
Table 1.

Frequency of Species Identified by Direct Observation, Animal Prints/Tracks, Live Trap or Game Camera

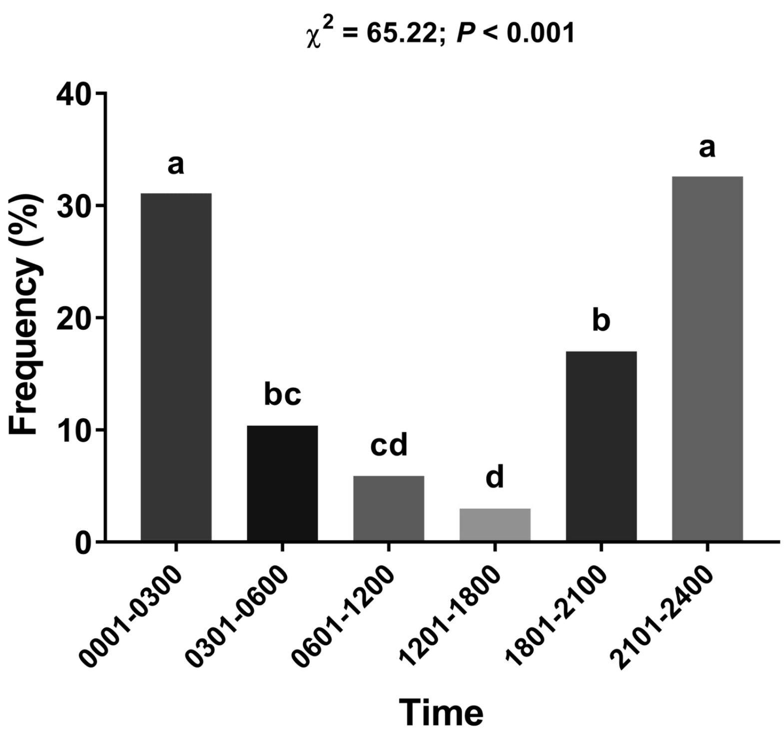
Species Name	Common Name	Total Identified (All Methods)	IUCN Red List Classification, Population Trend
Dasypus novemcinctus	Nine-banded Armadillo	58	Least Concern, Stable
Agouti paca	Paca, Gibnut	43	Least Concern, Stable
Leopardus pardalis	Ocelot	1	Least Concern, Decreasing
Dasyprocta punctata	Agouti	13	Least Concern, Stable
Conepatus semistriatus	Striped Hog-nosed Skunk	2	Least Concern, Unknown
Philander opossum	Gray Four-eyed Opossum	2	Least Concern, Stable
Tapirus bairdii	Baird's Tapir, Mountain Cow	9	Endangered, Decreasing
Pecari tajacu	Collared Peccary	2	Least Concern, Stable
Puma concolor	Puma, Mountain Lion	3	Least Concern, Decreasing
Alouatta pigra	Yucatan Black Howler Monkey	4	Endangered, Decreasing
Sqiuris deppei	Deppe's Squirrel	3	Least Concern, Stable
Potos flavus	Kinkajou	2	Least Concern, Decreasing
Nasua narica	Coati Mundi, Quash	4	Least Concern, Decreasing
Sigmodon hispidus	Hispid Cotton Rat	1	Least Concern, Increasing
Mazama americana	Red Brocket Deer	1	Deficient Data, Unknown
Leopardus wiedii	Margay	1	Near Threatened, Decreasing

Note. Red List Classification and Population Trend data provided by IUCN Red List, 2019.

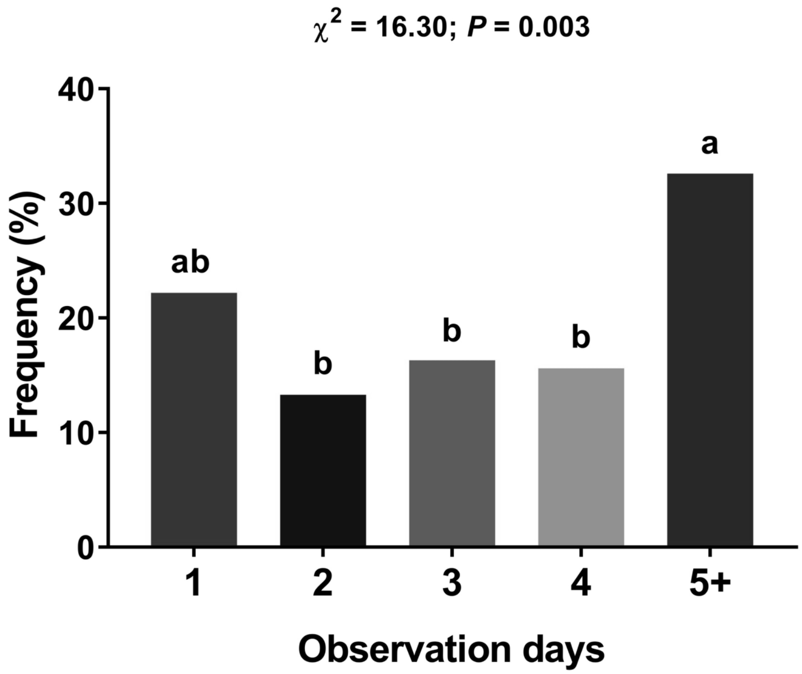
Graphs



Graph 1. Total frequency of photo captures on game cameras by species.



Graph 2. Frequency of photo captures on game cameras versus time of day.



Graph 3. Frequency of photo captures on game cameras versus days since placement.

Figures

Figure 1. Map of Belize (Billy Barquedier outlined in red), provided by Protected Planet (2019)

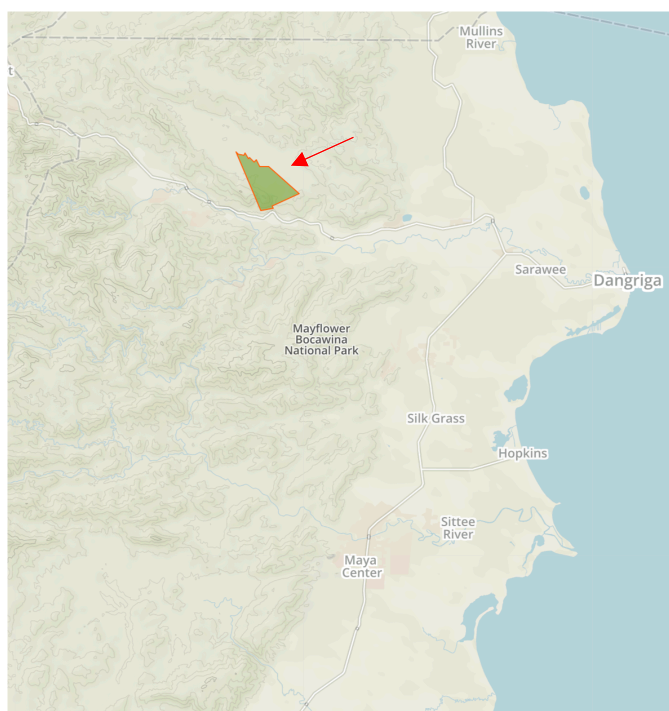


Figure 2. Map of Belize (Billy Barquedier outlined in red), provided by Protected Planet (2019)

Figure 3. Game camera photo of collared peccaries (*Pecari tajacu*

Figure 4. Game camera photos of pumas (*Puma concolor)*

Figure 5. Game camera photos of Baird's tapirs (*Tapirus bairdii)*

Figure 6. Game camera photos of striped hog-nosed skunks (*Conepatus semistriatus)*

Figure 7. Game camera photos of gray four-eyed opossums (*Philander opossum)*

Figure 8. Game camera photo of nine-banded armadillo (*Dasypus novemcinctus)*

Figure 9. Game camera photo of pacas (*Agouti paca)*

Figure 10. Game camera photo of agouti (*Dasyprocta punctata)*

Figure 11. Game camera photo (viewed through camera screen) of ocelot (*Leopardus pardalis)*

Figure 12. Game camera photo of margay (*Leopardus wiedii)*

Figure 13. Digital camera photos of hispid cotton rat (*Sigmodon hispidus)*

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